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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07H

A2

(11) International Publication Number: **WO 00/34294**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/US99/29400

(22) International Filing Date: 10 December 1999 (10.12.99)

(30) Priority Data:
60/111,826 11 December 1998 (11.12.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: TUMOR NECROSIS FACTOR RECEPTOR HOMOLOGUE–1 ("TRH1")

(57) Abstract

The present invention discloses the identification of the new human tumor necrosis factor receptor homologue ("TRH1"), as identified by its nucleic acid and amino acid sequences disclosed herein. The invention also includes methods of using the nucleic acid sequence, the TRH1 protein, a monoclonal antibody specific for the novel tumor necrosis factor receptor, a ligand for the novel tumor necrosis factor receptor, and fusion proteins comprising all or a portion of the TRH1 disclosed herein.

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TUMOR NECROSIS FACTOR RECEPTOR HOMOLOGUE-1 ("TRH1")

Background of the Invention

The tumor necrosis factor receptor ("TNFr") superfamily consists primarily of transmembrane proteins that elicit signal transduction in a variety of cells. The TNFr superfamily of cell surface receptors includes TNFr1, TNFr2, Fas, 4-1BB, OX-40, DR4, DR5 and others. These molecules contain extracellular cysteine-rich TNFr domains, a transmembrane domain, and an intracellular cytoplasmic tail which mediates receptor interactions with intracellular signaling molecules. Members of this family mediate diverse biological events including cell activation, proliferation, differentiation, and apoptosis. (See, e.g., Smith, C.A., et al., (1994) Cell, 76:959-962, incorporated herein by reference).

Recently, a novel soluble member was described, called osteoprotegrin, which contains four cysteine-rich TNFr domains but lacks transmembrane and cytoplasmic domains. This molecule has been shown to regulate osteoclast differentiation and therefore may play a role in regulating bone mass during development and in the mature animal. (Simonet, W.S., et al., (1997) Cell, 89:309-319, incorporated herein by reference).

Because of the diverse biological activities of members of the TNFr superfamily and their close relationship to immune cell function (see, e.g., US Patent 5,670,319 to Goeddel et al., incorporated herein by reference), those skilled in the art are interested in identifying novel members of this family to: (1) better understand the mechanisms underlying immune cell function; (2) regulate or control immune reactions or disease; and (3) screen for compounds effective in modulating the activity of TNFr's.

Summary of the Invention

The present invention includes a novel TNFr homologue 1 ("TRH1") and the cDNA encoding said TRH1. The nucleotide sequence of the isolated cDNA is disclosed herein along with the deduced amino acid sequence. The cDNA gene has

been deposited with the American Type Culture Collection and given the Accession Number ATCC _____ .

The present inventors sequenced the cDNA encoding the novel TRH1 and determined the primary sequence of the deduced protein. The novel TRH1 has homology to known TNFr sequences.

The TRH1 of the present invention can be produced by: (1) inserting the cDNA of the disclosed TRH1 into an appropriate expression vector; (2) transfecting the expression vector into an appropriate transfection host(s); (3) growing the transfected host(s) in appropriate culture media; and (4) purifying the receptor protein from the culture media.

The present invention therefore provides a purified and isolated nucleic acid molecule, preferably a DNA molecule, having a sequence which codes for a TRH1, or an oligonucleotide fragment of the nucleic acid molecule which is unique to the TRH1 of the invention. In a preferred embodiment of the invention, the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:1. In another preferred embodiment, the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:2. In still another preferred embodiment the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:3. In still another preferred embodiment of the present invention the purified and isolated nucleic acid molecule has the nucleotide sequence as shown in SEQ ID NO:4.

The invention also contemplates a double stranded nucleic acid molecule comprising a nucleic acid molecule of the invention or an oligonucleotide fragment thereof hydrogen bonded to a complementary nucleotide base sequence.

The terms "isolated and purified nucleic acid" and "substantially pure nucleic acid", e.g., substantially pure DNA, refer to a nucleic acid molecule which is one or both of the following: (1) not immediately contiguous with either one or both of the sequences, e.g., coding sequences, with which it is immediately contiguous (i.e., one at the 5' end and one at the 3'end) in the naturally occurring genome of the organism from which the nucleic acid is derived; or (2) which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated

into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Substantially pure or isolated and purified DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional TRH1/TNFr sequence.

The present invention provides in one embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:5; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which exhibit at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions. In a particular embodiment, the fragment is a sequence encoding a TRH1 having the amino acid sequence as shown in SEQ ID NO:7 (encompassing from amino acid number 42 to 655 of Figure 2) and sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity thereto.

The degree of homology (percent identity) between a native and a mutant sequence may be determined, for example, by comparing the two sequences using computer programs commonly employed for this purpose. One suitable program is the GAP computer program described by Devereux et al., (1984) *Nucl. Acids Res.* 12:387. The GAP program utilizes the alignment method of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:433, as revised by Smith and Waterman (1981) *Adv. Appl. Math.* 2:482. Briefly, the GAP program defines percent identity as the number of aligned symbols (i.e., nucleotides or amino acids) which are identical, divided by the total number of symbols in the shorter of the two sequences.

As used herein the term "stringent conditions" encompasses conditions known in the art under which a nucleotide sequence will hybridize to an isolated and purified nucleic acid molecule comprising a sequence encoding a protein having the amino acid sequence as shown herein, or to (b) a nucleic acid sequence complementary to

(a). Screening polynucleotides under stringent conditions may be carried out according to the method described in Nature, 313:402-404 (1985). Polynucleotide sequences capable of hybridizing under stringent conditions with the polynucleotides of the present invention may be, for example, allelic variants of the disclosed DNA sequences, or may be derived from other sources. General techniques of nucleic acid hybridization are disclosed by Sambrook et al., "Molecular Cloning: A Laboratory Manual", 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1984); and by Haymes et al., "Nucleic Acid Hybridization: A Practical Approach", IRL Press, Washington, D.C. (1985), which references are incorporated herein by reference.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:6; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding a polypeptide having the amino acid sequence as shown in SEQ ID NO:8 (Gln42 through Pro351 of Figure 2); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention also provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:1; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:2; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:3; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:4; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention additionally covers nucleic acid molecules of the present invention having one or more structural mutations including replacement, deletion or insertion mutations. For example, a signal peptide may be deleted, or conservative amino acid substitutions may be made to generate a protein that is still biologically competent or active.

The invention further contemplates a recombinant molecule comprising a nucleic acid molecule of the present invention or an oligonucleotide fragment thereof and an expression control sequence operatively linked to the nucleic acid molecule or oligonucleotide fragment. A transformant host cell including a recombinant molecule of the invention is also provided.

In another aspect, the invention features a cell or purified preparation of cells which include a novel gene encoding a TRH1 of the present invention, or which otherwise misexpresses a gene encoding a TRH1 of the present invention. The cell

preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a TRH1 transgene, e.g., a heterologous form of a TRH1 gene, e.g., a gene derived from humans (in the case of a non-human cell). The TRH1 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpresses an endogenous TRH1 gene, e.g., a gene that expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or misexpressed TRH1 alleles for use in drug screening.

Still further, the invention provides plasmids which comprise the nucleic acid molecules of the invention.

The present invention also includes a novel TRH1 of the present invention, or an active part thereof. A biologically competent or active form of the protein or part thereof is also referred to herein as an "active TRH1 or part thereof".

The invention further contemplates antibodies having specificity against an epitope of the TRH1 of the present invention or part of the protein. These antibodies may be polyclonal or monoclonal. The antibodies may be labeled with a detectable substance and they may be used, for example, to detect the novel TRH1 of the invention in tissue and cells. Additionally, the antibodies of the present invention, or portions thereof, may be used to make targeted antibodies that destroy TRH1 expressing cells (e.g., antibody-toxin fusion proteins, or radiolabelled antibodies).

The invention also permits the construction of nucleotide probes which encode part or all of the novel TRH1 protein of the invention or a part of the protein. Thus, the invention also relates to a probe comprising a nucleotide sequence coding for a protein, which displays the properties of the novel TRH1 of the invention or a peptide unique to the protein. The probe may be labeled, for example, with a detectable (e.g., radioactive) substance and it may be used to select from a mixture of nucleotide sequences a nucleotide sequence coding for a protein which displays the properties of the novel TRH1 of the invention.

The present invention also provides a transgenic non-human animal (e.g., a rodent, e.g., a mouse or a rat, a rabbit or a pig) or embryo all of whose germ cells and

somatic cells contain a recombinant molecule of the invention, preferably a recombinant molecule comprising a nucleic acid molecule of the present invention encoding the TRH1 of the invention or part thereof. The recombinant molecule may comprise a nucleic acid sequence encoding the TRH1 of the present invention with a structural mutation, or may comprise a nucleic acid sequence encoding the TRH1 of the invention or part thereof and one or more regulatory elements which differ from the regulatory elements that drive expression of the native protein. In another preferred embodiment, the animal has a TRH1 gene which is misexpressed or not expressed, e.g., a knockout. Such transgenic animals can serve as a model for studying disorders which are related to mutated or misexpressed TRH1 of the present invention.

The invention still further provides a method for identifying a substance which is capable of binding the novel TRH1 of the invention, comprising reacting the novel TRH1 of the invention or part of the protein under conditions which permit the formation of a complex between the substance and the novel TRH1 protein or part of the protein, and assaying for substance-TRH1 complexes, for free substance, for non-complexed TRH1, or for activation of the TRH1.

An embodiment of the invention provides a method for identifying ligands which are capable of binding to the novel TRH1 protein of the invention, isoforms thereof, or part of the protein, said method comprising reacting the novel TRH1 protein of the invention, isoforms thereof, or part of the protein, with at least one ligand which potentially is capable of binding to the protein, isoform, or part of the protein, under conditions which permit the formation of ligand-receptor protein complexes, and assaying for ligand-receptor protein complexes, for free ligand, for non-complexed TRH1 protein, or for activation of the TRH1. In a preferred embodiment of the method, ligands are identified which are capable of binding to and activating the novel TRH1 protein of the invention, isoforms thereof, or part of the protein.

The invention also relates to a method for assaying a medium for the presence of an agonist or antagonist of the interaction of the novel TRH1 protein and a substance which is capable of binding the TRH1, said method comprising providing a

known concentration of the TRH1, reacting the TRH1 with a substance which is capable of binding to the TRH1 and a suspected agonist or antagonist under conditions which permit the formation of substance-TRH1 complexes, and assaying for substance-TRH1 complexes, for free substance, for non-complexed TRH1, or for activation of the TRH1.

The invention further provides a method for identifying a substance which is capable of binding to an activated TRH1 protein of the present invention or an isoform or a part of the protein, said method comprising reacting an activated TRH1 of the present invention, or an isoform or part of the protein, with at least one substance which potentially can bind with the TRH1, isoform or part of the protein, under conditions which permit the formation of substance-activated TRH1 complexes, and assaying for substance-TRH1 complexes, for free substance, or for non-complexed TRH1. The method may be used to identify intracellular ligands containing proteins which bind to an activated TRH1 protein of the present invention or parts thereof, or intracellular ligands which may be affected in other ways by the activated TRH1 of the invention.

Also included within the scope of the present invention is a composition which includes the TRH1 of the present invention, a fragment thereof (or a nucleic acid encoding said TRH1 or fragment thereof) and one or more additional components, e.g., a carrier, diluent or solvent. The additional component can be one which renders the composition useful for in vitro, in vivo, pharmaceutical or veterinary use.

In another aspect, the present invention relates to a method of treating a mammal, e.g., a human, at risk for a disorder, e.g., a disorder characterized by aberrant or unwanted level or biological activity of the TRH1 of the present invention, or characterized by an aberrant or unwanted level of a ligand that specifically binds to the TRH1 of the present invention. For example, the TRH1 of the present invention may be useful to leach out or block a ligand which is found to bind to the TRH1 of the present invention. Encompassed within the scope of the invention is a soluble form of the TRH1 of the present invention, e.g., a fragment of the receptor, that may be used to inhibit activation of the receptor by binding to the ligand a polypeptide of the

present invention and preventing the ligand from interacting with membrane bound TRH1.

Also within the scope of the present invention are fusion proteins comprising all or a portion of the TRH1 of the present invention. Preferably, the fusion protein comprises all or a portion of the extracellular region of the TRH1 of the present invention as shown in SEQ ID NO:8. All or a portion of the extracellular portion of the TRH1 of the present invention may be attached to another molecule or polypeptide, e.g., a hinge and/or constant region of an immunoglobulin ("Ig") protein.

The primary object of the present invention is the identification of the new human TRH1, as identified by its sequence disclosed herein. Additional objects of the invention are the methods of using the cDNA, the TRH1 protein, the monoclonal antibody specific for the novel TRH1, fusion proteins comprising a portion of the TRH1 protein of the present invention, and a ligand for the novel TRH1 as described above.

Brief Description of the Figures

Figure 1 shows the DNA sequences of TRH1 cDNA clones 2733717 (Figure 1A; SEQ ID NO:1) and 2098183 (Figure 1B; SEQ ID NO:11). These represent two of the cDNA clones that were identified by searches of the Incyte LifeSeq database.

Figure 2A-2D gives the deduced amino acid sequence of TRH1 (SEQ ID NO:5).

Figure 3 shows the results of Northern blot analysis of the tissue distribution of TRH1 mRNA. Filters containing polyA enriched RNA from multiple tissues (A, B, C, D) were hybridized with radiolabeled TRH1 cDNA probe. The sources of RNAs are listed across the tops of each panel and the sizes of RNA size standards in kb are shown by arrows on the left side of each panel. A mRNA of approximately 4.2 kb (3.5 kb in testis) hybridized with the TRH1 probe in many tissues.

Figure 4 demonstrates the production of TRH1 Ig fusion proteins. COS cells were transiently transfected with plasmids encoding the extracellular region of TRH1 fused to the hinge, CH2, and CH3 domains of mouse IgG2a (mIg) or human IgG1

(hIg). Fusion proteins from the supernatants of transfected cells that were pulsed with ^{35}S-methionine were purified by protein A affinity chromatography and subjected to SDS-PAGE analysis and autoradiography. The apparent masses of molecular weight standards in kDa are shown to the left of the panel.

Figure 5 shows the alignment of the peptide sequences of TRH1 with osteoprotegerin ("OPG") and TNF receptor type II ("TNFR2"). The extracellular region of TRH1 is aligned with the cysteine rich motifs of OPG and TNFR2 that are characteristic of TNFR family members. Aligned residues are: TRH1 50-213 (SEQ ID NO:12); OPG 24-187 (SEQ ID NO:13) from GenPept accession number 2072185; and TNFR2 38-202 (SEQ ID NO:14) from GenPept accession number 1469541. Residues that are strictly conserved between the three proteins are in bold lettering and all cysteine residues are underlined. TRH1 shares 34% identity with OPG and 39% identity with TNFR2 in the aligned extracellular regions.

Detailed Description of the Invention

In order to identify novel members of the tumor necrosis factor receptor ("TNFR") superfamily, BLAST (basic local alignment sequence tool) searches of EST (expressed sequence tag) databases were performed with peptide sequences of known TNFR family members. Two cDNA clones in the Incyte LifeSeq database were identified. One clone was derived from a kidney tumor cDNA library while the other was isolated from a teratocarcinoma cell line that had neural differentiation characteristics. They were obtained from Incyte and sequenced.

Since they did not contain the complete coding region, they were used in further BLAST searches to identify two additional clones, one from a brain tumor cDNA library and the other from an ovarian tumor library. These two cDNA clones of TNFR homologue 1 ("TRH1") were sequenced in entirety on both strands by the fluorescent dideoxy chain termination method. INCYTE cDNA clones 2733717 and 2098183 (Figure 1) both contain the complete peptide encoding sequences for TRH1 (Figure 2). Although both clones encode identical proteins, their 5' and 3' untranslated regions are not identical. There are at least two possible translation start sites in these clones (Met-1 and Met-25) (Figure 2). The predicted mature peptide

sequence begins at Gln42. Overall, the cDNA's encode for a 655 residue type 1 membrane protein. After cleavage of the predicted signal peptide, the 614 amino acid mature protein contains a 310 residue extracellular region (SEQ ID NO:8), a transmembrane spanning segment, and a 285 amino acid cytoplasmic region. The predicted molecular mass of the mature protein before posttranslational modification is approximately 68 kDa. Comparison of the extracellular region of TRH1 to other members of the TNFR superfamily reveals that it is most closely related to osteoprotegerin (Simonet, *supra*) and TNFr2 (see Figure 5).

A preferred nucleic acid sequence encoding a TRH1 of the present invention comprises nucleotides 52 through 2016 (SEQ ID NO:2) of the following SEQ ID NO:1:

```
ATTCGGCTGC CCCGCGCGCC CCGGGCGCCC CTGCGAGTCC CCGGTTCAGC C
51
ATG GGG ACC TCT CCG AGC AGC AGC ACC GCC CTC GCC TCC TGC
93
AGC CGC ATC GCC CGC CGA GCC ACA GCC ACG ATG ATC GCG GGC
135
TCC CTT CTC CTG CTT GGA TTC CTT AGC ACC ACC ACA GCT CAG
177
CCA GAA CAG AAG GCC TCG AAT CTC ATT GGC ACA TAC CGC CAT
219
GTT GAC CGT GCC ACC GGC CAG GTG CTA ACC TGT GAC AAG TGT
261
CCA GCA GGA ACC TAT GTC TCT GAG CAT TGT ACC AAC ACA AGC
303
CTG CGC GTC TGC AGC AGT TGC CCT GTG GGG ACC TTT ACC AGG
345
CAT GAG AAT GGC ATA GAG AAA TGC CAT GAC TGT AGT CAG CCA
387
TGC CCA TGG CCA ATG ATT GAG AAA TTA CCT TGT GCT GCC TTG
429
ACT GAC CGA GAA TGC ACT TGC CCA CCT GGC ATG TTC CAG TCT
471
AAC GCT ACC TGT GCC CCC CAT ACG GTG TGT CCT GTG GGT TGG
513
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GGT GTG CGG AAG AAA GGG ACA GAG ACT GAG GAT GTG CGG TGT
555
AAG CAG TGT GCT CGG GGT ACC TTC TCA GAT GTG CCT TCT AGT
597
GTG ATG AAA TGC AAA GCA TAC ACA GAC TGT CTG AGT CAG AAC
639
CTG GTG GTG ATC AAG CCG GGG ACC AAG GAG ACA GAC AAC GTC
681
TGT GGC ACA CTC CCG TCC TTC TCC AGC TCC ACC TCA CCT TCC
723
CCT GGC ACA GCC ATC TTT CCA CGC CCT GAG CAC ATG GAA ACC
765
CAT GAA GTC CCT TCC TCC ACT TAT GTT CCC AAA GGC ATG AAC
807
TCA ACA GAA TCC AAC TCT TCT GCC TCT GTT AGA CCA AAG GTA
849
CTG AGT AGC ATC CAG GAA GGG ACA GTC CCT GAC AAC ACA AGC
891
TCA GCA AGG GGG AAG GAA GAC GTG AAC AAG ACC CTC CCA AAC
933
CTT CAG GTA GTC AAC CAC CAG CAA GGC CCC CAC CAC AGA CAC
975
ATC CTG AAG CTG CTG CCG TCC ATG GAG GCC ACT GGG GGC GAG
1017
AAG TCC AGC ACG CCC ATC AAG GGC CCC AAG AGG GGA CAT CCT
1059
AGA CAG AAC CTA CAC AAG CAT TTT GAC ATC AAT GAG CAT TTG
1101
CCC TGG ATG ATT GTG CTT TTC CTG CTG CTG GTG CTT GTG GTG
1143
ATT GTG GTG TGC AGT ATC CGG AAA AGC TCG AGG ACT CTG AAA
1185
AAG GGG CCC CGG CAG GAT CCC AGT GCC ATT GTG GAA AAG GCA
1227
GGG CTG AAG AAA TCC ATG ACT CCA ACC CAG AAC CGG GAG AAA
1269

TGG ATC TAC TAC TGC AAT GGC CAT GGT ATC GAT ATC CTG AAG
1311
CTT GTA GCA GCC CAA GTG GGA AGC CAG TGG AAA GAT ATC TAT
1353
CAG TTT CTT TGC AAT GCC AGT GAG AGG GAG GTT GCT GCT TTC
1395
TCC AAT GGG TAC ACA GCC GAC CAC GAG CGG GCC TAC GCA GCT
1437
CTG CAG CAC TGG ACC ATC CGG GGC CCC GAG GCC AGC CTC GCC
1479
CAG CTA ATT AGC GCC CTG CGC CAG CAC CGG AGA AAC GAT GTT
1521
GTG GAG AAG ATT CGT GGG CTG ATG GAA GAC ACC ACC CAG CTG
1563
GAA ACT GAC AAA CTA GCT CTC CCG ATG AGC CCC AGC CCG CTT
1605
AGC CCG AGC CCC ATC CCC AGC CCC AAC GCG AAA CTT GAG AAT
1647
TCC GCT CTC CTG ACG GTG GAG CCT TCC CCA CAG GAC AAG AAC
1689
AAG GGC TTC TTC GTG GAT GAG TCG GAG CCC CTT CTC CGC TGT
1731
GAC TCT ACA TCC AGC GGC TCC TCC GCG CTG AGC AGG AAC GGT
1773
TCC TTT ATT ACC AAA GAA AAG AAG GAC ACA GTG TTG CGG CAG
1815
GTA CGC CTG GAC CCC TGT GAC TTG CAG CCT ATC TTT GAT GAC
1857
ATG CTC CAC TTT CTA AAT CCT GAG GAG CTG CGG GTG ATT GAA
1899
GAG ATT CCC CAG GCT GAG GAC AAA CTA GAC CGG CTA TTC GAA
1941
ATT ATT GGA GTC AAG AGC CAG GAA GCC AGC CAG ACC CTC CTG
1983
GAC TCT GTT TAT AGC CAT CTT CCT GAC CTG CTG **TAG**AACATAG
2026 GGATACTGCA TTCTGGAAAT TACTCAATTT AGTGGCAGGG
TGGTTTTTTA 2076 ATTTTCTTCT GTTTCTGATT TTTGTTGTTT

GGGGTGTGTG TGTGTGTTTG 2126 TGTGTGTGTG TGTGTGTGTG
TGTGTGTGTG TTTAACAGAG AATATGGCCA 2176 GTGCTTGAGT
TCTTTCTCCT TCTCTCTCTC TCTTTTTTTT TTAAATAACT 2226
CTTCTGGGAA GTTGGTTTAT AAGCCTTTGC CAGGTGTAAC TGTTGTGAAA
2276 TACCCACCAC TAAAGTTTTT TAAGTTCCAT ATTTTCTCCA
TTTTGCCTTC 2326 TTATGTATTT TCAAGATTAT TCTGTGCACT
TTAAATTTAC TTAACTTACC 2376 ATAAATGCAG TGTGACTTTT
CCCACACACT GGATTGTGAG GCTCTTAACT 2426 TCTTAAAAGT
ATAATGGCAT CTTGTGAATC CTATAAGCAG TCTTTATGTC 2476
TCTTAACATT CACACCTACT TTTTAAAAAC AAATATTATT ACTATTTTTA
2526 TTATTGTTTG TCCTTTATAA ATTTTCTTAA AGATTAAGAA
AATTTAAGAC 2576 CCCATTGAGT TACTGTAATG CAATTCAACT
TTGAGTTATC TTTTAAATAT 2626 GTCTTGTATA GTTCATATTC
ATGGCTGAAA CTTGACCACA CTATTGCTGA 2676 TTGTATGGTT
TTCACCTGGA CACCGTGTAG AATGCTTGAT TACTTGTACT 2726
CTTCTTATGC TAATATGCTC TGGGCTGGAG AAATGAAATC CTCAAGCCAT
2776 CAGGATTTGC TATTTAAGTG GCTTGACAAC TGGGCCACCA
AAGAACTTGA 2826 ACTTCACCTT TTAGGATTTG AGCTGTTCTG
GAACACATTG CTGCACTTTG 2876 GAAAGTCAAA ATCAAGTGCC
AGTGGCGCCC TTTCCATAGA GAATTTGCCC 2926 AGCTTTGCTT
TAAAAGATGT CTTGTTTTTT ATATACACAT AATCAATAGG 2976
TCCAATCTGC TCTCAAGGCC TTGGTCCTGG TGGGATTCCT TCACCAATTA
3026 CTTTAATTAA AAATGGCTGC AACTGTAAGA ACCCTTGTCT
GATATATTTG 3076 CAACTATGCT CCCATTTACA AATGTACCTT
CTAATGCTCA GTTGCCAGGT 3126 TCCAATGCAA AGGTGGCGTG
GACTCCCTTT GTGTGGGTGG GGTTTGTGGG 3176 TAGTGGTGAA
GGACCGATAT CAGAAAAATG CCTTCAAGTG TACTAATTTA 3226
TTAATAAACA TTAGGTGTTT GTTAAAAAAA AAATGGTTAA TAAAAAAAAG
3276
G
3277

The deduced amino acid sequence of a TRH1 of the present invention comprises the following SEQ ID NO:5:

MGTSPSSSTA LASCSRIARR ATATMIAGSL LLLGFLSTTT AQPEQKASNL
50 IGTYRHVDRA TGQVLTCDKC PAGTYVSEHC TNTSLRVCSS CPVGTFTRHE
100 NGIEKCHDCS QPCPWPMIEK LPCAALTDRE CTCPPGMFQS

NATCAPHTVC 150 PVGWGVRKKG TETEDVRCKQ CARGTFSDVP
SSVMKCKAYT DCLSQNLVVI 200 KPGTKETDNV CGTLPSFSSS
TSPSPGTAIF PRPEHMETHE VPSSTYVPKG 250 MNSTESNSSA
SVRPKVLSSI QEGTVPDNTS SARGKEDVNK TLPNLQVVNH 300
QQGPHHRHIL KLLPSMEATG GEKSSTPIKG PKRGHPRQNL HKHFDINEHL
350 PWMIVLFLLL VLVVIVVCSI RKSSRTLKKG PRQDPSAIVE
KAGLKKSMTP 400 TQNREKWIYY CNGHGIDILK LVAAQVGSQW
KDIYQFLCNA SEREVAAFSN 450 GYTADHERAY AALQHWTIRG
PEASLAQLIS ALRQHRRNDV VEKIRGLMED 500 TTQLETDKLA
LPMSPSPLSP SPIPSPNAKL ENSALLTVEP SPQDKNKGFF 550
VDESEPLLRC DSTSSGSSAL SRNGSFITKE KKDTVLRQVR LDPCDLQPIF
600 DDMLHFLNPE ELRVIEEIPQ AEDKLDRLFE IIGVKSQEAS
QTLLDSVYSH 650
LPDLL*
655

The predicted signal peptide of the above TRH1 protein spans residues 1-41. The two potential translational start sites are Met1 and Met25 (bold). After cleavage of the predicted signal peptide, the mature protein (SEQ ID NO:7) begins at Gln42 (bold and underlined). The predicted membrane spanning region is underlined.

The present invention relates to the nucleic acid sequence or a fragment thereof (referred to herein as a "polynucleotide") of the novel TRH1 as shown above (SEQ ID NO:2), as well as to the amino acid sequence of the TNFr (SEQ ID NO:5), and biologically active portions thereof. SEQ ID NO:3 shows the nucleic acid sequence of a portion (Met25 through Leu655) of the TRH1 receptor of the present invention; SEQ ID NO:6 shows the amino acid sequence of a portion of the TRH1 receptor sequence. SEQ ID NO:8 shows the amino acid sequence of the extracellular portion of the TRH1 protein of the present invention.

The present invention further relates to variants of the hereinabove described nucleic acid sequence which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of SEQ ID NO:5 or the polypeptide encoded by the cDNA of the deposited clones. The variants of the nucleic acid sequence may be naturally occurring variants of the nucleic acid sequence or non-naturally occurring variants of the nucleic acid sequence.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in SEQ ID NO:5, or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of SEQ ID NO:5 or the polypeptide encoded by the cDNA of the deposited clones. Specifically included within the scope of the present invention is a polynucleotide as shown in SEQ ID NO:3, encoding a 631 amino acid protein as shown in SEQ ID NO:6 (Met25 through Leu655); and a polynucleotide as shown in SEQ ID NO:4, encoding a 614 amino acid protein as shown in SEQ ID NO:7 (Gln42 through Leu655). Such nucleotide variants include deletion variants, substitution variants and addition or insertion (splice) variants.

The polynucleotides may also encode for a soluble form of the TRH1 receptor polypeptide of the present invention which is the extracellular portion of the polypeptide which has been cleaved from the transmembrane and intracellular domain of the full length polypeptide of the present invention. A preferred polynucleotide encodes the polypeptide of SEQ ID NO:8, which represents the extracellular portion of the TRH1 of the present invention.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Fragments of the full length gene of the present invention may be used as hybridization probes for a cDNA library to isolate the full length gene and to isolate other genes which have a high sequence similarity to a gene of the present invention or similar biological activity. Probes of this type preferably have at least between 20 and 30 bases, and may contain, for example, 50 or more bases. The probes may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene of the present invention including regulatory and promoter regions, exons, and introns.

The present invention further relates to polynucleotides that hybridize to the polynucleotide sequences disclosed herein, if there is at least 80%, preferably at least

90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the polynucleotides described herein.

Alternatively the polynucleotide may have at least 20 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotide of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, for example for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus the present invention is directed to polynucleotides having at least 80% identity, preferably at least 90% and more preferably at least 95% identity to a polynucleotide of the present invention, including polynucleotides encoding the polypeptide of SEQ ID NO:5, as well as fragments thereof, which fragments have at least 20 or 30 bases, and preferably at least 50 bases, and to polypeptides encoded by such polynucleotides.

The present invention further relates to a tumor necrosis factor receptor homologue polypeptide, TRH1, which has the deduced amino acid sequence as shown in SEQ ID NO:5 (Figure 2), or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide. Encompassed within the scope of the present invention are polypeptides as shown in SEQ ID NO:6, SEQ ID NO:7 (the mature protein) and SEQ ID NO:8 (the extracellular portion of the TRH1 protein).

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-Organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence(s) of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with

Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-1-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Other analogs within the invention are those with modifications which increase protein or peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the protein or peptide sequence. Also included are analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., ß or γ amino acids.

In terms of general utility of the novel TRH1 protein of the present invention, the expression pattern of the protein and homology to members of the TNFr superfamily indicate that this novel TNFr homologue plays a role in cellular function, including but not limited to cell activation, proliferation, differentiation, and apoptosis. Similar to other receptor systems being investigated (e.g., CD40/CD40L, 4-1BB/4-1BBL, Fas/FasL) it is contemplated by the present invention that the interaction between the novel TNFr protein of the present invention and intracellular signaling molecules and/or its potential co-receptor may serve as a novel target for immunosuppressive, antiinflammatory and/or immunostimulatory drug development.

Gene constructs of the present invention can also be used as part of a gene therapy protocol to deliver nucleic acids encoding the TRH1 of the present invention, or an agonist or antagonist form of a TRH1 protein or peptide. The invention features expression vectors for in vivo transfection and expression of a TRH1. Expression constructs of the TRH1 of the present invention, may be administered in any biologically effective carrier, e.g., any formulation or composition capable of effectively delivering the TRH1 gene to cells in vivo. Approaches include insertion of

any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

Analogs of the novel TRH1 of the present invention are also within the scope of the present invention. Analogs can differ from the naturally occurring TRH1 of the present invention in amino acid sequence or in ways that do not involve sequence, or both. Non-sequence modifications include in vivo or in vitro chemical derivitization of the TRH1 of the present invention. Non-sequence modifications include changes in acetylation, methylation, phosphorylation, carboxylation, or glycosylation.

Preferred analogs include the novel TRH1 of the present invention (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the biological activity of the TRH1 of the present invention. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative amino acid substitutions can be taken from the table below.

Table 1

Conservative amino acid replacements

For Amino Acid	Code	Replace with any of:
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, ß-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val

the subject gene in viral vectors including recombinant retroviruses, adenoviruses, adeno-associated viruses, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; an advantage of infection of cells with a viral vector is that a large proportion of the targeted cells can receive the nucleic acid. Several viral delivery systems are known in the art and can be utilized by one practicing the present invention.

In addition to viral transfer methods, non-viral methods may also be employed to cause expression of the TRH1 in the tissue of an animal. Most non-viral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes. DNA of the present invention may also be introduced to cell(s) by direct injection of the gene construct or electroporation.

In clinical settings, the gene delivery systems for the therapeutic TRH1 gene can be introduced into a patient by any of a number of methods, each of which is known in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof.

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Another aspect of the invention relates to the use of an isolated nucleic acid in "antisense" therapy. As used herein, "antisense" therapy refers to administration or in situ generation of oligonucleotides or their derivatives which specifically hybridize under cellular conditions, with the cellular mRNA and/or genomic DNA encoding the

TRH1 of the present invention so as to inhibit expression of the encoded protein, e.g., by inhibiting transcription and/or translation. In general, "antisense" therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

Fragments of the TNFr of the present invention are also within the scope of Applicant's invention. Fragments of the protein can be produced in several ways, e.g., recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Digestion with "end-nibbling" endonucleases can thus generate DNA's which encode an array of fragments. DNA's which encode fragments of the TRH1 protein can also be generated by random shearing, restriction digestion or a combination of the above-discussed methods.

Fragments can also be chemcially synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry.

Amino acid sequence variants of the TRH1 protein of the present invention can be prepared by random mutagenesis of DNA which encodes a protein or a particular domain or region of the protein. Useful methods are known in the art, e.g., PCR mutagenesis and saturation mutagenesis. A library of random amino acid sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotides sequences, a process known and practiced by those skilled in the art.

Non-random or directed mutagenesis techniques can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions of residues of the known amino acid sequence of the TRH1 protein of the present invention. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conserved amino acids then with more radical choices depending upon results achieved; (2) deleting the target residue; or (3) inserting residues of the same or a different class (e.g., hydrophobic or hydrophilic) adjacent to the located site, or a combination of options (1)-(3). Alanine scanning mutagenesis is a useful method for

identification of certain functional residues or regions of a desired protein that are preferred locations or domains for mutagenesis. Oligonucleotide-mediated mutagenesis, cassette mutagenesis, and combinatorial mutagenesis are useful methods known to those skilled in the art for preparing substitution, deletion, and insertion variants of DNA.

The present invention also relates to methods of screening. Various techniques are known in the art for screening generated mutant gene products. Techniques for screening large gene libraries often include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the genes under conditions in which detection of a desired activity, e.g., in this case binding of a ligand to the TRH1 of the present invention. Techniques known in the art are amenable to high through-put analysis for screening large numbers of sequences created, e.g., by random mutagenesis techniques.

Two hybrid assays can be used to identify fragments or analogs of a protein or peptide which bind to the TRH1 of the present invention. These may include agonists or antagonists. In one approach to screening assays, the candidate protein or peptides are displayed on the surface of a cell or viral particle, and the ability of particular cells or viral particles to bind an appropriate receptor protein via the displayed product is detected in a "panning assay". In a similar fashion, a detectably labeled ligand can be used to score for potentially functional peptide homologues. Fluorescently labeled ligands, e.g., receptors, can be used to detect homologue which retain ligand-binding activity. The use of fluorescently labeled ligand allows cells to be visually inspected and separated under fluorescence microscope or to be separated by a fluorescence-activated cell sorter.

High through-put assays can be followed by secondary screens in order to identify further biological activities which will, e.g., allow one skilled in the art to differentiate agonists from antagonists. The type of a secondary screen used will depend on the desired activity that needs to be tested. For example, an assay can be developed in which the ability to inhibit an interaction between the TRH1 of the present invention and its respective ligand can be used to identify antagonists from a

group of peptide fragments isolated through one of the primary screens. Therefore, methods for generating fragments and analogs and testing them for activity are known in the art. Once a sequence of interest is identified, it is routine for one skilled in the art to obtain agonistic or antagonistic analogs, fragments, and/or ligands.

Drug screening assays are also provided in the present invention. By producing purified and recombinant TRH1 of the present invention, or fragments thereof, one skilled in the art can use these to screen for drugs which are either agonists or antagonists of the normal cellular function or their role in cellular signaling. In one embodiment, the assay evaluates the ability of a compound to modulate binding between the TRH1 of the present invention and a naturally occurring ligand. The term "modulating" encompasses enhancement, diminishment, activation or inactivation of the TRH1 receptor. Assays useful to identify ligands to the TRH1 receptor of the present invention, including peptides, proteins, small molecules, and antibodies that are capable of binding to the TRH1 receptor are encompassed herein. A variety of assay formats will suffice and are known by those skilled in the art.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as primary screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound.

Also within the scope of the present invention is a process for modulating the TRH1 of the present invention. The term "modulating" encompasses enhancement, diminishment, activation or inactivation of the TRH1 receptor. Ligands to the TRH1 receptor of the present invention, including peptides, proteins, small molecules, and antibodies, that are capable of binding to the TRH1 receptor and modulating its activity are encompasses herein. These compounds are useful in modulating the activity of the TRH1 receptor and in treating TRH1-associated disorders. "TRH1-associated disorders" refers to any disorder or disease state in which the TRH1 protein

plays a regulatory role in the metabolic pathway of that disorder or disease. Such disorders or diseases may include rheumatoid arthritis and transplant rejection. As used herein the term "treating" refers to the alleviation of symptoms of a particular disorder in a patient, the improvement of an ascertainable measurement associated with a particular disorder, or the prevention of a particular immune, inflammatory or cellular response (such as transplant rejection).

The invention also includes antibodies specifically reactive with the TRH1 of the present invention, or a portion thereof. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard known procedures. A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques known in the art. An immunogenic portion of the TRH1 of the present invention can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum.

The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with the TRH1 of the present invention. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as whole antibodies. For example, F(ab')2 fragments can be generated by treating antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibody of the present invention is further intended to include chimeric and humanized molecules that recognize and bind to the TRH1 of the present invention.

Both monoclonal and polyclonal antibodies directed against the TRH1 of the present invention, and antibody fragments such as Fab', sFv and F(ab')2, can be used to block the action of the TRH1 of the present invention and allow study of the role of a particular TRH1 of the present invention. Alternatively, such antibodies can be used therapeutically to block the TRH1 of the present invention in a subject mammal, e.g., a human. In a preferred embodiment a therapeutic compositions comprising an antibody of the present invention can also comprise a pharmaceutically acceptable carrier, solvent or diluent, and be administered by systems known in the art.

Antibodies of the present invention may also be useful as potential agonists of the TRH1 of the present invention. Such agonistic antibodies tend to aggregate and crosslink the receptor, which induces signaling, proliferation, differentiation and/or cell death (apoptosis).

Antibodies which specifically bind to the TRH1 of the present invention, or fragments thereof, can also be used in immunohistochemical staining of tissue samples in order to evaluate the abundance and pattern expression of the TRH1 of the present invention. Antibodies can be used diagnostically in immunoprecipitation, immunoblotting, and enzyme linked immunosorbent assay (ELISA) to detect and evaluate levels of the TRH1 of the present invention in tissue or bodily fluid.

EXAMPLES

The following examples are included for understanding the present invention and are not intended to limit the scope of Applicants invention.

Example 1: Northern blot analysis

In order to determine the tissue distribution of TRH1 mRNA, Northern blot analyses of filters containing polyA enriched RNA from multiple tissues and cells lines was performed. Multiple tissue Northern blots were obtained commercially (Clontech, Palo Alto, CA). A fragment of clone 2098183 was labeled with P^{32}-dCTP using the random prime labeling kit (Boerhinger) and then hybridized to filters that had been prehybridized for 3 hours at 65° with prehybridization/hybridization solution (0.5 M $NaHPO_4$ pH 7.2, 1 mM EDTA, 1% bovine serum albumin, 7% sodium dodecyl sulfate). Hybridization was performed overnight at 65°C. The filters were subsequently washed with low stringency wash buffer (0.5% bovine serum albumin, 1 mM EDTA, 40 mM $NaHPO_4$ pH 7.2, 5% sodium dodecyl sulfate) for 30 minutes at room temperature and 30 minutes at 42°C. The filters were then washed with high stringency wash buffer (40 mM $NaHPO_4$ pH 7.2, 1 mM EDTA, 1% sodium dodecyl sulfate) for 30 minutes at 42°C and 30 minutes 55°C. The filters were then exposed to X-ray film at -70°C with intensifying screens and then developed.

From Northern analysis, an approximately 4.2 kb mRNA is expressed in multiple tissues and cell lines. TRH1 is most highly expressed in thymus, prostate, small intestine (Fig 3A), lymph node (Fig 3B), HeLa (cervical adenocarcinoma), Molt4 (T cell lymphoma), SW480 (colorectal carcinoma), A549 (lung carcinoma), and G361 (melanoma) (Fig 3C), and heart, brain, placenta, kidney, and pancreas (Fig 3D). Messenger RNAs of approximately 4.2 and 3.5 kb also hybridized in the testis (Fig 3A).

Example 2: Production of TRH1 immunoglobulin (Ig) fusion proteins

Complementary DNA fragments encoding the extracellular region of TRH1 (Met1 to Pro351) (although the mature protein would be expected to not have the signal peptide encompassing amino acids 1-41, it was encoded in the fragment used to construct the fusion protein) were produced by polymerase chain reaction (PCR) methodology using cDNA clone 2098183 as a template with oligonucleotides containing the appropriate endonuclease restriction sites to mediate fusion with human IgG1 or mouse IgG2a expression cassettes as already described (Aruffo et al., (1990) *Cell* 61:1303-1313; Bowen et al., (1996) *JBC* 271(29):17390-17396). The sense oligonucletide was (GCC AAG CTT CGA GTC CCC GGT TCA GCC ATG (SEQ ID NO:9)) and encoded a HindIII restriction site and bases 34 to 54 of clone 2733717. The antisense oligonucletide (GCG TGG ATC CGG ATG TCC CCT CTT GGG GCC (SEQ ID NO:10)) contained a BamHI restriction site and encoded the reverse complement of bases 1039 to 1058 of clone 2733717. These oligonucleotides were used to amplify cDNA sequences that encode amino acids Met1 through Pro336 of TRH1 (Fig 2). The PCR was performed with high fidelity Taq polymerase (Boerhinger) with the following cycling conditions: 1 cycle: 94°C 3 min; 40 cycles: 94° 1 min., 60° 1 min., 72° 2.5 min.; 1 cycle: 72° 5 min.

Proteins were produced by transient transfection of COS cells that had been pulsed 16h with ^{35}S-Methionine (Amersham Corp.) 24h after transfection. The fusion proteins were purified with Protein A Sepharose and subsequently analyzed by SDS-PAGE and autoradiography (Fig 4).

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

We claim:

1. A purified and isolated nucleic acid sequence encoding all or a portion of a tumor necrosis factor receptor homologue, said tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:5.

2. The nucleic acid sequence of claim 1 comprising the nucleic acid sequence as shown in SEQ ID NO:2.

3. A purified and isolated nucleic acid sequence encoding all or a portion of a tumor necrosis factor receptor homologue, said tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:6.

4. The nucleic acid sequence of claim 3 comprising the nucleic acid sequence as shown in SEQ ID NO:3.

5. A purified and isolated nucleic acid sequence encoding all or a portion of a tumor necrosis factor receptor homologue, said tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:7.

6. The nucleic acid sequence of claim 5 comprising the nucleic acid sequence as shown in SEQ ID NO:4.

7. An expression vector comprising a nucleic acid molecule as claimed in claim 1, 2, 3, 4, 5, or 6 and an expression control sequence operatively linked to the nucleic acid molecule.

8. A transformant host cell including an expression vector comprising a nucleic acid molecule as claimed in claim 1, 2, 3, 4, 5 or 6 and an expression control sequence operatively linked to the nucleic acid molecule.

9. A purified and isolated nucleic acid sequence comprising the complement of the nucleic acid sequence of claim 1, 2, 3, 4, 5 or 6.

10. A tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:5.

11. A tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:6.

12. A tumor necrosis factor receptor homologue comprising the amino acid sequence as shown in SEQ ID NO:7.

13. A method of producing a tumor necrosis factor receptor homologue, said method comprising the steps of:

a) inserting a nucleic acid sequence according to claim 1, 2, 3, 4, 5 or 6 encoding said tumor necrosis factor receptor homologue into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host cell,

c) growing said transfected host cells in an appropriate culture media, and

d) purifying the tumor necrosis factor receptor from said culture media.

14. An isolated nucleic acid sequence which hybridizes under stringent conditions to the nucleic acid sequence of claim 2, 4 or 6.

15. An antibody specific for the tumor necrosis factor receptor homologue as claimed in claim 10.

16. The antibody of claim 15 wherein said antibody is a monoclonal antibody.

17. An antibody specific for the tumor necrosis factor receptor homologue as claimed in claim 11.

18. The antibody of claim 17 wherein said antibody is a monoclonal antibody.

19. An antibody specific for the tumor necrosis factor receptor homologue as claimed in claim 12.

20. The antibody of claim 19 wherein said antibody is a monoclonal antibody.

21. The tumor necrosis factor receptor homologue of claim 10, produced by:

a) inserting a nucleic acid sequence encoding said tumor necrosis factor receptor homologue into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host cell,

c) growing said transfected host cells in an appropriate culture media, and

d) purifying the tumor necrosis factor receptor from said culture media.

22. The tumor necrosis factor receptor homologue of claim 11, produced by:

a) inserting a nucleic acid sequence encoding said tumor necrosis factor receptor homologue into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host cell,

c) growing said transfected host cells in an appropriate culture media, and

d) purifying the tumor necrosis factor receptor from said culture media.

23. A method for identifying a ligand which is capable of binding to the tumor necrosis factor receptor homologue of claim 10, or to a part of said tumor necrosis factor receptor homologue, said method comprising the steps of:

(a) reacting said tumor necrosis factor receptor homologue, or part of said tumor necrosis factor receptor homologue, with said ligand which potentially is capable of binding to the tumor necrosis factor receptor homologue or part of said tumor necrosis factor receptor homologue, under conditions which permit the formation of ligand-tumor necrosis factor receptor homologue complexes; and

(b) assaying for ligand-tumor necrosis factor receptor homologue complexes, for free ligand, for non-complexed tumor necrosis factor receptor homologue, or for activation of the tumor necrosis factor receptor homologue.

24. A method for identifying a ligand which is capable of binding to the tumor necrosis factor receptor homologue of claim 11, or to a part of said tumor necrosis factor homologue, said method comprising the steps of:

(a) reacting said tumor necrosis factor receptor homologue, or part of said tumor necrosis factor receptor homologue, with said ligand which potentially is capable of binding to the tumor necrosis factor receptor homologue or part of said

tumor necrosis factor receptor homologue, under conditions which permit the formation of ligand-tumor necrosis factor receptor homologue complexes; and

(b) assaying for ligand-tumor necrosis factor receptor homologue complexes, for free ligand, for non-complexed tumor necrosis factor receptor homologue, or for activation of the tumor necrosis factor receptor homologue.

25. A method for identifying a ligand which is capable of binding to the tumor necrosis factor receptor homologue of claim 12, or to a part of said tumor necrosis factor receptor homologue, said method comprising the steps of:

(a) reacting said tumor necrosis factor receptor homologue, or part of said tumor necrosis factor receptor homologue, with said ligand which potentially is capable of binding to the tumor necrosis factor receptor homologue or part of said tumor necrosis factor receptor homologue, under conditions which permit the formation of ligand-tumor necrosis factor receptor homologue complexes; and

(b) assaying for ligand-tumor necrosis factor receptor homologue complexes, for free ligand, for non-complexed tumor necrosis factor receptor homologue, or for activation of the tumor necrosis factor receptor homologue.

26. A fusion protein comprising all or a portion of the tumor necrosis factor receptor homologue as shown in SEQ ID NO:5, attached to a second polypeptide.

27. The fusion protein of claim 26 comprising the extracellular portion of the tumor necrosis factor receptor homologue as shown in SEQ ID NO:5 attached to all or a portion of the hinge and/or constant region of a human IgG molecule.

28. A fusion protein comprising all or a portion of the polypeptide as shown in SEQ ID NO:8.

FIG. 1A

cDNA clone 2733717

ATTCGGCTGCCCCGCGCGCCCCGGGCGCCCCTGCGAGTCCCCGGTTCAGCCATGGGGACC
TCTCCGAGCAGCAGCACCGCCCTCGCCTCCTGCAGCCGCATCGCCCGCCGAGCCACAGCC
ACGATGATCGCGGGCTCCCTTCTCCTGCTTGGATTCCTTAGCACCACCACAGCTCAGCCA
GAACAGAAGGCCTCGAATCTCATTGGCACATACCGCCATGTTGACCGTGCCACCGGCCAG
GTGCTAACCTGTGACAAGTGTCCAGCAGGAACCTATGTCTCTGAGCATTGTACCAACACA
AGCCTGCGCGTCTGCAGCAGTTGCCCTGTGGGGACCTTTACCAGGCATGAGAATGGCATA
GAGAAATGCCATGACTGTAGTCAGCCATGCCCATGGCCAATGATTGAGAAATTACCTTGT
GCTGCCTTGACTGACCGAGAATGCACTTGCCCACCTGGCATGTTCCAGTCTAACGCTACC
TGTGCCCCCCATACGGTGTGTCCTGTGGGTTGGGGTGTGCGGAAGAAAGGGACAGAGACT
GAGGATGTGCGGTGTAAGCAGTGTGCTCGGGGTACCTTCTCAGATGTGCCTTCTAGTGTG
ATGAAATGCAAAGCATACACAGACTGTCTGAGTCAGAACCTGGTGGTGATCAAGCCGGGG
ACCAAGGAGACAGACAACGTCTGTGGCACACTCCCGTCCTTCTCCAGCTCCACCTCACCT
TCCCCTGGCACAGCCATCTTTCCACGCCCTGAGCACATGGAAACCCATGAAGTCCCTTCC
TCCACTTATGTTCCCAAAGGCATGAACTCAACAGAATCCAACTCTTCTGCCTCTGTTAGA
CCAAAGGTACTGAGTAGCATCCAGGAAGGGACAGTCCCTGACAACACAAGCTCAGCAAGG
GGGAAGGAAGACGTGAACAAGACCCTCCCAAACCTTCAGGTAGTCAACCACCAGCAAGGC
CCCCACCACAGACACATCCTGAAGCTGCTGCCGTCCATGGAGGCCACTGGGGGCGAGAAG
TCCAGCACGCCCATCAAGGGCCCCAAGAGGGGACATCCTAGACAGAACCTACACAAGCAT
TTTGACATCAATGAGCATTTGCCCTGGATGATTGTGCTTTTCCTGCTGCTGGTGCTTGTG
GTGATTGTGGTGTGCAGTATCCGGAAAAGCTCGAGGACTCTGAAAAAGGGGCCCCGGCAG
GATCCCAGTGCCATTGTGGAAAAGGCAGGGCTGAAGAAATCCATGACTCCAACCCAGAAC
CGGGAGAAATGGATCTACTACTGCAATGGCCATGGTATCGATATCCTGAAGCTTGTAGCA
GCCCAAGTGGGAAGCCAGTGGAAAGATATCTATCAGTTTCTTTGCAATGCCAGTGAGAGG
GAGGTTGCTGCTTTCTCCAATGGGTACACAGCCGACCACGAGCGGGCCTACGCAGCTCTG
CAGCACTGGACCATCCGGGGCCCCGAGGCCAGCCTCGCCCAGCTAATTAGCGCCCTGCGC
CAGCACCGGAGAAACGATGTTGTGGAGAAGATTCGTGGGCTGATGGAAGACACCACCCAG
CTGGAAACTGACAAACTAGCTCTCCCGATGAGCCCCAGCCCGCTTAGCCCGAGCCCCATC
CCCAGCCCCAACGCGAAACTTGAGAATTCCGCTCTCCTGACGGTGGAGCCTTCCCCACAG
GACAAGAACAAGGGCTTCTTCGTGGATGAGTCGGAGCCCCTTCTCCGCTGTGACTCTACA
TCCAGCGGCTCCTCCGCGCTGAGCAGGAACGGTTCCTTTATTACCAAAGAAAAGAAGGAC
ACAGTGTTGCGGCAGGTACGCCTGGACCCCTGTGACTTGCAGCCTATCTTTGATGACATG
CTCCACTTTCTAAATCCTGAGGAGCTGCGGGTGATTGAAGAGATTCCCCAGGCTGAGGAC
AAACTAGACCGGCTATTCGAAATTATTGGAGTCAAGAGCCAGGAAGCCAGCCAGACCCTC
CTGGACTCTGTTTATAGCCATCTTCCTGACCTGCTGTAGAACATAGGGATACTGCATTCT
GGAAATTACTCAATTTAGTGGCAGGGTGGTTTTTTAATTTTCTTCTGTTTCTGATTTTTG
TTGTTTGGGGTGTGTGTGTGTTTGTGTGTGTGTGTGTGTGTGTGTTTA
ACAGAGAATATGGCCAGTGCTTGAGTTCTTTCTCCTTCTCTCTCTCTCTTTTTTTTTTAA
ATAACTCTTCTGGGAAGTTGGTTTATAAGCCTTTGCCAGGTGTAACTGTTGTGAAATACC
CACCACTAAAGTTTTTTAAGTTCCATATTTTCTCCATTTTGCCTTCTTATGTATTTTCAA
GATTATTCTGTGCACTTTAAATTTACTTAACTTACCATAAATGCAGTGTGACTTTTCCCA
CACACTGGATTGTGAGGCTCTTAACTTCTTAAAAGTATAATGGCATCTTGTGAATCCTAT
AAGCAGTCTTTATGTCTCTTAACATTCACACCTACTTTTTAAAAACAAATATTATTACTA
TTTTTATTATTGTTTGTCCTTTATAAATTTTCTTAAAGATTAAGAAAATTTAAGACCCCA
TTGAGTTACTGTAATGCAATTCAACTTTGAGTTATCTTTTAAATATGTCTTGTATAGTTC
ATATTCATGGCTGAAACTTGACCACACTATTGCTGATTGTATGGTTTTCACCTGGACACC
GTGTAGAATGCTTGATTACTTGTACTCTTCTTTATGCTAATATGCTCTGGGCTGGAGAAAT
GAAATCCTCAAGCCATCAGGATTTGCTATTTAAGTGGCTTGACAACTGGGCCACCAAAGA
ACTTGAACTTCACCTTTTAGGATTTGAGCTGTTCTGGAACACATTGCTGCACTTTGGAAA
GTCAAAATCAAGTGCCAGTGGCGCCCTTTCCATAGAGAATTTGCCCAGCTTTGCTTTAAA
AGATGTCTTGTTTTTTATATACACATAATCAATAGGTCCAATCTGCTCTCAAGGCCTTGG
TCCTGGTGGGATTCCTTCACCAATTACTTTAATTAAAAATGGCTGCAACTGTAAGAACCC
TTGTCTGATATATTTGCAACTATGCTCCCATTTACAAATGTACCTTCTAATGCTCAGTTG
CCAGGTTCCAATGCAAAGGTGGCGTGGACTCCCTTTGTGTGGGTGGGGTTTGTGGGTAGT
GGTGAAGGACCGATATCAGAAAAATGCCTTCAAGTGTACTAATTTATTAATAAACATTAG
GTGTTTGTTAAAAAAAAAATGGTTAATAAAAAAAAGG

FIG. 1B

cDNA clone 2098183

CCACGCGTCCGGCCATGGGGACCTCTCCGAGCAGCAGCACCGCCCTCGCCTCCTGCAGCC
GCATCGCCCGCCGAGCCACAGCCACGATGATCGCGGGCTCCCTTCTCCTGCTTGGATTCC
TTAGCACCACCACAGCTCAGCCAGAACAGAAGGCCTCGAATCTCATTGGCACATACCGCC
ATGTTGACCGTGCCACCGGCCAGGTGCTAACCTGTGACAAGTGTCCAGCAGGAACCTATG
TCTCTGAGCATTGTACCAACACAAGCCTGCGCGTCTGCAGCAGTTGCCCTGTGGGGACCT
TTACCAGGCATGAGAATGGCATAGAGAAATGCCATGACTGTAGTCAGCCATGCCCATGGC
CAATGATTGAGAAATTACCTTGTGCTGCCTTGACTGACCGAGAATGCACTTGCCCACCTG
GCATGTTCCAGTCTAACGCTACCTGTGCCCCCCATACGGTGTGTCCTGTGGGTTGGGGTG
TGCGGAAGAAAGGGACAGAGACTGAGGATGTGCGGTGTAAGCAGTGTGCTCGGGGTACCT
TCTCAGATGTGCCTTCTAGTGTGATGAAATGCAAAGCATACACAGACTGTCTGAGTCAGA
ACCTGGTGGTGATCAAGCCGGGGACCAAGGAGACAGACAACGTCTGTGGCACACTCCCGT
CCTTCTCCAGCTCCACCTCACCTTCCCCTGGCACAGCCATCTTTCCACGCCCTGAGCACA
TGGAAACCCATGAAGTCCCTTCCTCCACTTATGTTCCCAAAGGCATGAACTCAACAGAAT
CCAACTCTTCTGCCTCTGTTAGACCAAAGGTACTGAGTAGCATCCAGGAAGGGACAGTCC
CTGACAACACAAGCTCAGCAAGGGGGAAGGAAGACGTGAACAAGACCCTCCCAAACCTTC
AGGTAGTCAACCACCAGCAAGGCCCCCACCACAGACACATCCTGAAGCTGCTGCCGTCCA
TGGAGGCCACTGGGGGCGAGAAGTCCAGCACGCCCATCAAGGGCCCCAAGAGGGGACATC
CTAGACAGAACCTACACAAGCATTTTGACATCAATGAGCATTTGCCCTGGATGATTGTGC
TTTTCCTGCTGCTGGTGCTTGTGGTGATTGTGGTGTGCAGTATCCGGAAAAGCTCGAGGA
CTCTGAAAAAGGGGCCCCGGCAGGATCCCAGTGCCATTGTGGAAAAGGCAGGGCTGAAGA
AATCCATGACTCCAACCCAGAACCGGGAGAAATGGATCTACTACTGCAATGGCCATGGTA
TCGATATCCTGAAGCTTGTAGCAGCCCAAGTGGGAAGCCAGTGGAAAGATATCTATCAGT
TTCTTTGCAATGCCAGTGAGAGGGAGGTTGCTGCTTTCTCCAATGGGTACACAGCCGACC
ACGAGCGGGCCTACGCAGCTCTGCAGCACTGGACCATCCGGGGCCCCGAGGCCAGCCTCG
CCCAGCTAATTAGCGCCCTGCGCCAGCACCGGAGAAACGATGTTGTGGAGAAGATTCGTG
GGCTGATGGAAGACACCACCCAGCTGGAAACTGACAAACTAGCTCTCCCGATGAGCCCCA
GCCCGCTTAGCCCGAGCCCCATCCCCAGCCCCAACGCGAAACTTGAGAATTCCGCTCTCC
TGACGGTGGAGCCTTCCCCACAGGACAAGAACAAGGGCTTCTTCGTGGATGAGTCGGAGC
CCCTTCTCCGCTGTGACTCTACATCCAGCGGCTCCTCCGCGCTGAGCAGGAACGGTTCCT
TTATTACCAAAGAAAAGAAGGACACAGTGTTGCGGCAGGTACGCCTGGACCCCTGTGACT
TGCAGCCTATCTTTGATGACATGCTCCACTTTCTAAATCCTGAGGAGCTGCGGGTGATTG
AAGAGATTCCCCAGGCTGAGGACAAACTAGACCGGCTATTCGAAATTATTGGAGTCAAGA
GCCAGGAAGCCAGCCAGACCCTCCTGGACTCTGTTTATAGCCATCTTCCTGACCTGCTGT
AGAACATAGGGATACTGCATTCTGGAAATTACTCAATTTAGTGGCAGGGTGGTTTTTTAA
TTTTCTTCTGTTTCTGATTTTTGTTGTTTGGGGTGTGTGTGTGTGTTTGTGTGTGTGTGT
GTGTGTGTGTGTGTGTGTGTTTAACAGAGAATATGGCCAGTGCTTGAGTTCTTTCTCC
TTCTCTCTCTCTCTTTTTTTTTTAAATAACTCTTCTGGGAAGTTGGTTTATAAGCCTTTG
CCAGGTGTAACTGTTGTGAAATACCCACCACTAAAGTTTTTTAAGTTCCATATTTTCTCC
ATTTTGCCTTCTTATGTATTTTCAAGATTATTCTGTGCACTTTAAATTTACTTAACTTAC
CATAAATGCAGTGTGACTTTTCCCACACACTGGATTGTGAGGCTCTTAACTTCTTAAAAG
TATAATGGCATCTTGTGAATCCTATAAGCAGTCTTTATGTCTCTTAACATTCACACCTAC
TTTTTAAAAACAAATATTATTACTATTTTTATTATTGTTTGTCCTTTATAAATTTTCTTA
AAGATTAAGAAAATTTAAGACCCCATTGAGTTACTGTAATGCAATTCAACTTTGAGTTAT
CTTTTAAATATGTCTTGTATAGTTCATATTCATGGCTGAAACTTGACCACACTATTGCTG
ATTGTATGGTTTTCACCTGGACACCGTGTAGAATGCTTGATTACTTGTACTCTTCTTATG
CTAATATGCTCTGGGCTGGAGAAATGAAATCCTCAAGCCATCAGGATTTGCTATTTAAGT
GGCTTGACAACTGGGCCACCAAAGAACTTGAACTTCACCTTTTAGGATTTGAGCTGTTCT
GGAACACATTGCTGCACTTTGGAAAGTCAAAATCAAGTGCCAGTGGCGCCCTTTCCATAG
AGAATTTGCCCAGCTTTGCTTTAAAAGATGTCTTGTTTTTTATATACACATAATCAATAG
GTCCAATCTGCTCTCAAGGCCTTGGTCCTGGTGGGATTCCTTCACCAATTACTTTAATTA
AAAATGGCTGCAACTGTAAGAACCCTTGTCTGATATATTTGCAACTATGCTCCCATTTAC
AAATGTACCTTCTAATGCTCAGTTGCCAGGTTCCAATGCAAAGGTGGCGTGGACTCCCTT
TGTGTGGGTGGGGTTTGTGGGTAGTGGTGAAGGACCGATATCAGAAAAATGCCTTCAAGT
GTACTAATTTATTAATAAACATTAGGTGTTTGTTAAAAAAAAAAATAAAAAAAAAG

FIG. 2A

```
                                                                  M  G  T
ATTCGGCTGCCCCGCGCGCCCCGGGCGCCCCTGCGAGTCCCCGGTTCAGCCATGGGGACC
        10        20        30        40        50        60

S  P  S  S  S  T  A  L  A  S  C  S  R  I  A  R  R  A  T  A
TCTCCGAGCAGCAGCACCGCCCTCGCCTCCTGCAGCCGCATCGCCCGCCGAGCCACAGCC
        70        80        90       100       110       120

T  M  I  A  G  S  L  L  L  L  G  F  L  S  T  T  T  A  Q  P
ACGATGATCGCGGGCTCCCTTCTCCTGCTTGGATTCCTTAGCACCACCACAGCTCAGCCA
       130       140       150       160       170       180

E  Q  K  A  S  N  L  I  G  T  Y  R  H  V  D  R  A  T  G  Q
GAACAGAAGGCCTCGAATCTCATTGGCACATACCGCCATGTTGACCGTGCCACCGGCCAG
       190       200       210       220       230       240

V  L  T  C  D  K  C  P  A  G  T  Y  V  S  E  H  C  T  N  T
GTGCTAACCTGTGACAAGTGTCCAGCAGGAACCTATGTCTCTGAGCATTGTACCAACACA
       250       260       270       280       290       300

S  L  R  V  C  S  S  C  P  V  G  T  F  T  R  H  E  N  G  I
AGCCTGCGCGTCTGCAGCAGTTGCCCTGTGGGGACCTTTACCAGGCATGAGAATGGCATA
       310       320       330       340       350       360

E  K  C  H  D  C  S  Q  P  C  P  W  P  M  I  E  K  L  P  C
GAGAAATGCCATGACTGTAGTCAGCCATGCCCATGGCCAATGATTGAGAAATTACCTTGT
       370       380       390       400       410       420

A  A  L  T  D  R  E  C  T  C  P  P  G  M  F  Q  S  N  A  T
GCTGCCTTGACTGACCGAGAATGCACTTGCCCACCTGGCATGTTCCAGTCTAACGCTACC
       430       440       450       460       470       480

C  A  P  H  T  V  C  P  V  G  W  G  V  R  K  K  G  T  E  T
TGTGCCCCCCCATACGGTGTGTCCTGTGGGTTGGGGTGTGCGGAAGAAAGGGACAGAGACT
       490       500       510       520       530       540

E  D  V  R  C  K  Q  C  A  R  G  T  F  S  D  V  P  S  S  V
GAGGATGTGCGGTGTAAGCAGTGTGCTCGGGGTACCTTCTCAGATGTGCCTTCTAGTGTG
       550       560       570       580       590       600

M  K  C  K  A  Y  T  D  C  L  S  Q  N  L  V  V  I  K  P  G
ATGAAATGCAAAGCATACACAGACTGTCTGAGTCAGAACCTGGTGGTGATCAAGCCGGGG
       610       620       630       640       650       660

T  K  E  T  D  N  V  C  G  T  L  P  S  F  S  S  S  T  S  P
ACCAAGGAGACAGACAACGTCTGTGGCACACTCCCGTCCTTCTCCAGCTCCACCTCACCT
       670       680       690       700       710       720

S  P  G  T  A  I  F  P  R  P  E  H  M  E  T  H  E  V  P  S
TCCCCTGGCACAGCCATCTTTCCACGCCCTGAGCACATGGAAACCCATGAAGTCCCTTCC
       730       740       750       760       770       780

S  T  Y  V  P  K  G  M  N  S  T  E  S  N  S  S  A  S  V  R
TCCACTTATGTTCCCAAAGGCATGAACTCAACAGAATCCAACTCTTCTGCCTCTGTTAGA
       790       800       810       820       830       840

P  K  V  L  S  S  I  Q  E  G  T  V  P  D  N  T  S  S  A  R
CCAAAGGTACTGAGTAGCATCCAGGAAGGGACAGTCCCTGACAACACAAGCTCAGCAAGG
       850       860       870       880       890       900

G  K  E  D  V  N  K  T  L  P  N  L  Q  V  V  N  H  Q  Q  G
GGGAAGGAAGACGTGAACAAGACCCTCCCAAACCTTCAGGTAGTCAACCACCAGCAAGGC
       910       920       930       940       950       960
```

FIG. 2B

```
P  H  H  R  H  I  L  K  L  L  P  S  M  E  A  T  G  G  E  K
CCCCACCACAGACACATCCTGAAGCTGCTGCCGTCCATGGAGGCCACTGGGGGCGAGAAG
      970        980        990       1000       1010       1020

S  S  T  P  I  K  G  P  K  R  G  H  P  R  Q  N  L  H  K  H
TCCAGCACGCCCATCAAGGGCCCCAAGAGGGGACATCCTAGACAGAACCTACACAAGCAT
      1030       1040       1050       1060       1070       1080

F  D  I  N  E  H  L  P  W  M  I  V  L  F  L  L  L  V  L  V
TTTGACATCAATGAGCATTTGCCCTGGATGATTGTGCTTTTCCTGCTGCTGGTGCTTGTG
      1090       1100       1110       1120       1130       1140

V  I  V  V  C  S  I  R  K  S  S  R  T  L  K  K  G  P  R  Q
GTGATTGTGGTGTGCAGTATCCGGAAAAGCTCGAGGACTCTGAAAAAGGGGCCCCGGCAG
      1150       1160       1170       1180       1190       1200

D  P  S  A  I  V  E  K  A  G  L  K  K  S  M  T  P  T  Q  N
GATCCCAGTGCCATTGTGGAAAAGGCAGGGCTGAAGAAATCCATGACTCCAACCCAGAAC
      1210       1220       1230       1240       1250       1260

R  E  K  W  I  Y  Y  C  N  G  H  G  I  D  I  L  K  L  V  A
CGGGAGAAATGGATCTACTACTGCAATGGCCATGGTATCGATATCCTGAAGCTTGTAGCA
      1270       1280       1290       1300       1310       1320

A  Q  V  G  S  Q  W  K  D  I  Y  Q  F  L  C  N  A  S  E  R
GCCCAAGTGGGAAGCCAGTGGAAAGATATCTATCAGTTTCTTTGCAATGCCAGTGAGAGG
      1330       1340       1350       1360       1370       1380

E  V  A  A  F  S  N  G  Y  T  A  D  H  E  R  A  Y  A  A  L
GAGGTTGCTGCTTTCTCCAATGGGTACACAGCCGACCACGAGCGGGCCTACGCAGCTCTG
      1390       1400       1410       1420       1430       1440

Q  H  W  T  I  R  G  P  E  A  S  L  A  Q  L  I  S  A  L  R
CAGCACTGGACCATCCGGGGCCCCGAGGCCAGCCTCGCCCAGCTAATTAGCGCCCTGCGC
      1450       1460       1470       1480       1490       1500

Q  H  R  R  N  D  V  V  E  K  I  R  G  L  M  E  D  T  T  Q
CAGCACCGGAGAAACGATGTTGTGGAGAAGATTCGTGGGCTGATGGAAGACACCACCCAG
      1510       1520       1530       1540       1550       1560

L  E  T  D  K  L  A  L  P  M  S  P  S  P  L  S  P  S  P  I
CTGGAAACTGACAAACTAGCTCTCCCGATGAGCCCCAGCCCGCTTAGCCCGAGCCCCATC
      1570       1580       1590       1600       1610       1620

P  S  P  N  A  K  L  E  N  S  A  L  L  T  V  E  P  S  P  Q
CCCAGCCCCAACGCGAAACTTGAGAATTCCGCTCTCCTGACGGTGGAGCCTTCCCCACAG
      1630       1640       1650       1660       1670       1680

D  K  N  K  G  F  F  V  D  E  S  E  P  L  L  R  C  D  S  T
GACAAGAACAAGGGCTTCTTCGTGGATGAGTCGGAGCCCCTTCTCCGCTGTGACTCTACA
      1690       1700       1710       1720       1730       1740

S  S  G  S  S  A  L  S  R  N  G  S  F  I  T  K  E  K  K  D
TCCAGCGGCTCCTCCGCGCTGAGCAGGAACGGTTCCTTTATTACCAAAGAAAAGAAGGAC
      1750       1760       1770       1780       1790       1800

T  V  L  R  Q  V  R  L  D  P  C  D  L  Q  P  I  F  D  D  M
ACAGTGTTGCGGCAGGTACGCCTGGACCCCTGTGACTTGCAGCCTATCTTTGATGACATG
      1810       1820       1830       1840       1850       1860

L  H  F  L  N  P  E  E  L  R  V  I  E  E  I  P  Q  A  E  D
CTCCACTTTCTAAATCCTGAGGAGCTGCGGGTGATTGAAGAGATTCCCCAGGCTGAGGAC
      1870       1880       1890       1900       1910       1920
```

FIG. 2C

```
K  L  D  R  L  F  E  I  I  G  V  K  S  Q  E  A  S  Q  T  L
AAACTAGACCGGCTATTCGAAATTATTGGAGTCAAGAGCCAGGAAGCCAGCCAGACCCTC
      1930       1940       1950       1960       1970       1980

L  D  S  V  Y  S  H  L  P  D  L  L  Stop
CTGGACTCTGTTTATAGCCATCTTCCTGACCTGCTGTAGAACATAGGGATACTGCATTCT
      1990       2000       2010       2020       2030       2040

GGAAATTACTCAATTTAGTGGCAGGGTGGTTTTTTAATTTTCTTCTGTTTCTGATTTTTG
      2050       2060       2070       2080       2090       2100

TTGTTTGGGGTGTGTGTGTGTTTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTTA
      2110       2120       2130       2140       2150       2160

ACAGAGAATATGGCCAGTGCTTGAGTTCTTTCTCCTTCTCTCTCTCTCTTTTTTTTTTAA
      2170       2180       2190       2200       2210       2220

ATAACTCTTCTGGGAAGTTGGTTTATAAGCCTTTGCCAGGTGTAACTGTTGTGAAATACC
      2230       2240       2250       2260       2270       2280

CACCACTAAAGTTTTTTAAGTTCCATATTTTCTCCATTTTGCCTTCTTATGTATTTTCAA
      2290       2300       2310       2320       2330       2340

GATTATTCTGTGCACTTTAAATTTACTTAACTTACCATAAATGCAGTGTGACTTTTCCCA
      2350       2360       2370       2380       2390       2400

CACACTGGATTGTGAGGCTCTTAACTTCTTAAAAGTATAATGGCATCTTGTGAATCCTAT
      2410       2420       2430       2440       2450       2460

AAGCAGTCTTTATGTCTCTTAACATTCACACCTACTTTTTAAAAACAAATATTATTACTA
      2470       2480       2490       2500       2510       2520

TTTTTATTATTGTTTGTCCTTTATAAATTTTCTTAAAGATTAAGAAAATTTAAGACCCCA
      2530       2540       2550       2560       2570       2580

TTGAGTTACTGTAATGCAATTCAACTTTGAGTTATCTTTTAAATATGTCTTGTATAGTTC
      2590       2600       2610       2620       2630       2640

ATATTCATGGCTGAAACTTGACCACACTATTGCTGATTGTATGGTTTTCACCTGGACACC
      2650       2660       2670       2680       2690       2700

GTGTAGAATGCTTGATTACTTGTACTCTTCTTATGCTAATATGCTCTGGGCTGGAGAAAT
      2710       2720       2730       2740       2750       2760

GAAATCCTCAAGCCATCAGGATTTGCTATTTAAGTGGCTTGACAACTGGGCCACCAAAGA
      2770       2780       2790       2800       2810       2820

ACTTGAACTTCACCTTTTAGGATTTGAGCTGTTCTGGAACACATTGCTGCACTTTGGAAA
      2830       2840       2850       2860       2870       2880

GTCAAAATCAAGTGCCAGTGGCGCCCTTTCCATAGAGAATTTGCCCAGCTTTGCTTTAAA
      2890       2900       2910       2920       2930       2940

AGATGTCTTGTTTTTTATATACACATAATCAATAGGTCCAATCTGCTCTCAAGGCCTTGG
      2950       2960       2970       2980       2990       3000

TCCTGGTGGGATTCCTTCACCAATTACTTTAATTAAAAATGGCTGCAACTGTAAGAACCC
      3010       3020       3030       3040       3050       3060

TTGTCTGATATATTTGCAACTATGCTCCCATTTACAAATGTACCTTCTAATGCTCAGTTG
      3070       3080       3090       3100       3110       3120
```

FIG. 2D

```
CCAGGTTCCAATGCAAAGGTGGCGTGGACTCCCTTTGTGTGGGTGGGGTTTGTGGGTAGT
      3130       3140       3150       3160       3170       3180

GGTGAAGGACCGATATCAGAAAAATGCCCTTCAAGTGTACTAATTTATTAATAAACATTAG
      3190       3200       3210       3220       3230       3240

GTGTTTGTTAAAAAAAAAATGGTTAATAAAAAAAAGG
      3250       3260       3270   3277
```

FIG. 3A

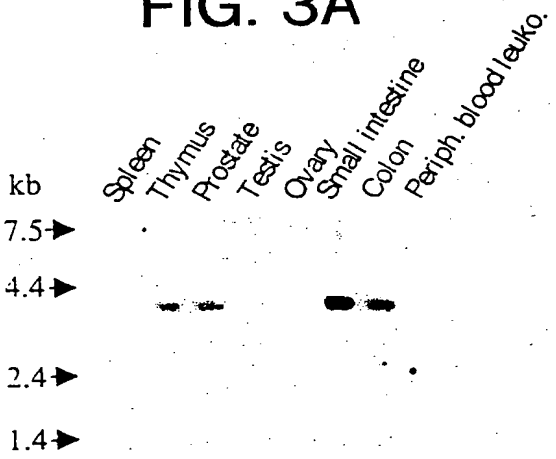

FIG. 3B

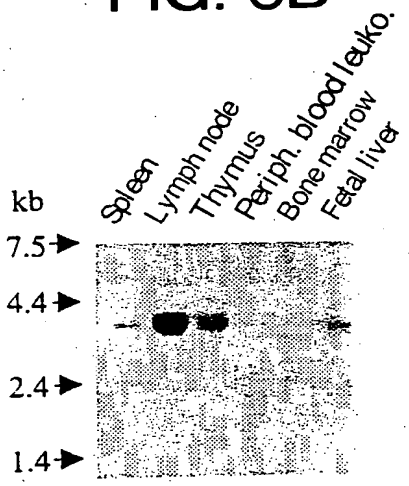

FIG. 3C

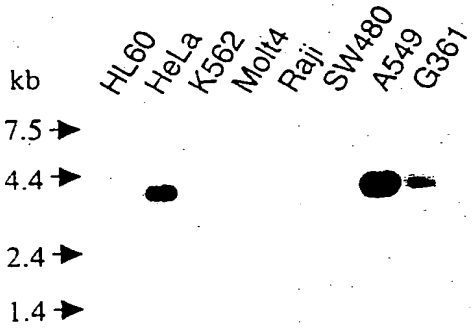

FIG. 3D

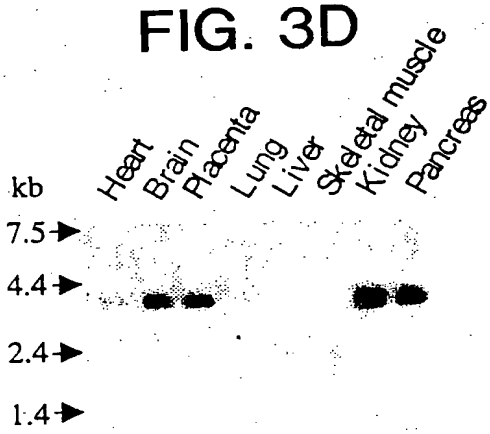

FIG. 4

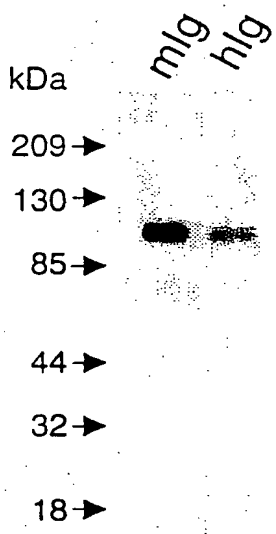

FIG. 5

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                                                                  60
TRH1    LIGTYRHVDR ATGQVLTCDK CPAGTYVSEH CTNTSLRVCS SCPVGTFTRH ENGIEKCHDC
OPG     FPPKYLHYDE ETSHQLLCDK CPPGTYLKQH CTAKWKTVCA PCPDHYYTDS WHTSDECLYC
TNFR2   STCRLREYYD QTA.QMCCSK CSPGQHAKVF CTKTSDTVCD SCEDSTYTQL WNWVPECLSC
                                                                 120
TRH1    SQPCPWPMIE KLPCAALTDR ECTCPPGMFQ SN.....AT CAPHTVCPVG WGVRKKGTET
OPG     SPVCKELQYV KQECNRTHNR VCECKEGRYL EI.....EF CLKHRSCPPG FGVVQAGTPE
TNFR2   GSRCSSDQVE TQACTREQNR ICTCRPGWYC ALSKQEGCRL CAPLRKCRPG FGVARPGTET
                                                                 170
TRH1    EDVRCKQCAR GTFSDVPSSV MKCKAYTDCL SQNLVVIKPG TKETDNVCGT
OPG     RNTVCKRCPD GFFSNETSSK APCRKHTNCS VFGLLLTQKG NATHDNICSG
TNFR2   SDVVCKPCAP GTFSNTTSST DICRPHQICN VVAI....PG NASRDAVCTS
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/47, C07H 21/04, C12N 15/12, 15/64, C12P 21/02 — A3

(11) International Publication Number: WO 00/34294

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/US99/29400

(22) International Filing Date: 10 December 1999 (10.12.99)

(30) Priority Data:
60/111,826 11 December 1998 (11.12.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
26 October 2000 (26.10.00)

(54) Title: TUMOR NECROSIS FACTOR RECEPTOR HOMOLOGUE–1 ("TRH1")

(57) Abstract

The present invention discloses the identification of the new human tumor necrosis factor receptor homologue ("TRH1"), as identified by its nucleic acid and amino acid sequences disclosed herein. The invention also includes methods of using the nucleic acid sequence, the TRH1 protein, a monoclonal antibody specific for the novel tumor necrosis factor receptor, a ligand for the novel tumor necrosis factor receptor, and fusion proteins comprising all or a portion of the TRH1 disclosed herein.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29400

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 530/350; 536/23.5; 435/320.1, 252.3, 69.1, 7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 435/320.1, 252.3, 69.1, 7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Commercial Sequence Databases, MPSRCH
Searches SEQ ID NOS: 1-8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 869 179 A1 (SMITHKLINE BEECHAM CORPORATION) 07 October 1998 (07.10.98), see entire document, especially SEQ ID NOS: 1 and 2, claims 1-11, 16, 18 and 20.	1-14, 21-25
X	Database GenEmbl, Direct Submission (Pathology, University of Michigan Medical School, Ann Arbor, MI, USA), AN AF068868, PAN et al. 'Identification and Functional Characterization of DR6, a Novel Death Domain-containing TNF Receptor.' Gene Sequence, 05 September 1998, see entire document.	1-14, 21-22
X	Database EST, AN AA351536, ADAMS et al.'Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence.' Gene Sequence, Nature. 21 April 1997, Vol. 377, No. 6547 Suppl. pages 3-174, see entire document.	1-6, 9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 APRIL 2000	Date of mailing of the international search report 13 JUN 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EILEEN B. O'HARA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29400

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database EST, AN AA155873, HILLIER et al. 'Generation and analysis of 280,000 human expressed sequence tags.' Gene Sequence, Genome Res. 11 December 1996, Vol. 6, No. 9, pages 807-828, see entire document.	1-6, 9
Y,P	WO 98/56892 A1 (HUMAN GENOME SCIENCES INC.) 17 December 1998 (17.12.98), see entire document, especially SEQ ID NOS; 1 and 2, and claims 1-4, 8, 11-21, 23-24 and page 49, line 24 to page 50, line 5.	1-14, 21, 26-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29400

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29400

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 14/47; C07

C07K 14/47; C07H 21/04; C12N 15/12, 15/64; C12P 21/02

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-14, 21, 22 and 26-28, drawn to nucleic acids,polypeptides encoded by the nucleic acids, vectors, host cells, and a recombinant method of making the polypeptides.
Group II, claim(s) 15-20, drawn to antibodies.
Group III, claim(s) 23-25, drawn to a method for identifying a ligand that binds to a polypeptide.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product, nucleic acids encoding TRH1, vectors, host cells, a recombinant method of producing TRH1, and TRH1 polypeptide. Further pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.